

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Marie Bendix HANSEN *et al.*

Title: **METHOD FOR HIGH THROUGHPUT VOLUMES IN THE
FRACTIONATION OF BIO-MOLECULES BY
CHROMATOGRAPHIC SYSTEMS**

Appl. No.: 10/548,403

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Examiner: Alexander D. Kim

Art Unit: 1656

Confirmation
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DECLARATION UNDER 37 C.F.R. § 1.132

I, Allan Otto Fog Lihme, declare as follows:

1. I am named as the inventor of the above-identified U.S. patent application, which I understand is under examination by the United States Patent and Trademark Office.
2. I have a masters degree in biochemistry from University of Copenhagen and more than 25 years of experience in the field of protein separation and analysis (hereafter, "the field"). I am currently the Technical Director at UpFront Chromatography A/S. My resume accompanies this declaration.
3. I have reviewed and believe that I understand those portions addressed below of an Official Action, dated January 12th, 2011. I also have reviewed and am familiar with the publications cited in this Official Action. I am an a named inventor on one of the cited publications, WO 02/096215.

Linear Flow Rate Calculations

4. In the Official Action, at pages 2-5, the examiner discusses chromatographic linear flow rates. At page 3 the examiner disputes certain linear flow rate calculations, which were presented previously, I understand, with regard to a characterization of Example 19 in WO 02/096215. I am familiar with the calculations and the characterization in question, and the work described in Example 19 was performed under my supervision.
5. With respect to the aforementioned calculations, the examiner states that the “applicants have divided the linear flow rate of 1628 cm/hr by 3 or 2 according to the number of column in series which is not an accurate liner flow rate calculation,” and that “the linaer flow rate is 1628 cm/hr regardless how long or how many column is attached.” Official Action at page 3 (emphasis added).
6. Thus, I understand the examiner to believe that the columns described in Example 19 were run in series. This is incorrect, however. The columns described in Example 19 actually were run in parallel, a fact that a person knowledgeable in the field would apprehend from the context of the example.
7. The text of WO 02/096215 states that Example 19 is a theoretical calculation and estimate of the production output in a larger plant (*i.e.*, a theoretical scale-up), using the described adsorbents and the “fractionation facility” (*i.e.*, the two-step process, column compositions and methods) described in the preceding Example 18. More specifically, Example 18 describes a two-step process, with one column used in “Step I” and one column used in “Step II.” The flow rates for each step are defined explicitly in Example 18: the load and wash flow rate for the single column of Step I is 900 cm/hr, and the elution flow rate is 450 cm/hr. The flow rate for the single column of Step II is 450 cm/hr for the whole process.
8. As noted, Example 19 provides theoretical calculations for a scale-up of the Example 18 process. Example 19 differs from Example 18 in that two columns are used in Step I and three columns are used in Step II. Example 19 clearly refers to the “fractionation facility” of Example 18. Accordingly, the flow rates employed in the calculations in Example 19 must be (and in fact are) the same

flow rates described in Example 18. Any other reading of the text would render meaningless the mention in Example 19 of the “fractionation facility” of Example 18.

9. In order for the flow rates to remain the same upon scale-up, per Example 19, the two columns in Step I, and the three columns in Step II, per Example 18, must have been configured in parallel. That is, a person knowledgeable in the field would readily appreciate that, when scaling up such a process, one must make larger columns and/or add more columns in parallel. Conversely, putting more columns in series and changing flow rates significantly would create in effect a different process and a different “fractionation facility,” not in keeping with what WO 02/096215 actually says.
10. Thus, the knowledgeable reader of WO 02/096215 would appreciate that Example 19 contemplates a scale-up of the process described in Example 18, where the columns were arranged in parallel and the flow rates were much lower than 1500 cm/hour.

Flickinger

11. At pages 9-11 of the Official Action, the examiner discusses the Flickinger reference, U.S. Patent No. 5,837,826. I understand the examiner to believe that Flickinger describes an expanded bed chromatographic method that will function at high flow rates (greater than 1500 cm/hr) and at high temperatures (greater than 45 °C).
12. I disagree and believe it is highly unlikely that the columns described in Flickinger could operate effectively, if at all, were they run at the flow rates and temperatures that characterize the invention of the application.
13. I have extensive experience with expanded bed chromatography (EBC). The success of EBC is dependent on many variables such as the type of solvent, type of adsorbent particles, temperatures, pH, and turbulent flow in the column, to name but a few.

14. Because the parameters and variables in EBC are complex and interlinked, I and others in the EBC area would be obliged to carry out an extensive program of research to balance the relevant parameters to increase flow rates from a known “high” working rate (*e.g.*, 220 cm/hr) to a much higher rate of 1500 cm/hr. In reviewing Flickinger, I noted that all of the actual examples are carried out at flow rates of at most 220 cm/hr (see Table 5). Which variables or parameters to change and precisely how to change them are not subject to a foregone conclusion with any EBC system. Increasing flow rate, especially to rates greater than 1500 cm/hr in an EBC system, is not simply a matter of “opening the tap.”
15. After reading Flickinger, I do not believe the EBC columns described in this reference could be run at 1500 cm/hr without substantial modification. The Flickinger column beds probably would destabilize at flow rates much faster than 220 cm/hr. Moreover, the likelihood of bed destabilization increases with increasing temperature. Accordingly, using higher flow rates, *e.g.*, 1500 cm/hr or greater, and higher temperatures as in the subject invention would very probably result in completely non-functional columns, at least in the absence of extensive experimentation and appropriate modification.
16. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of United States Code, and that such wilful false statements may jeopardize the validity of the above-referenced application, or any patent issuing thereof.

Date

Allan Otto Fog Lihme